Vectored ImmunoProphylaxis Protects Humanized Mice from Mucosal HIV Transmission

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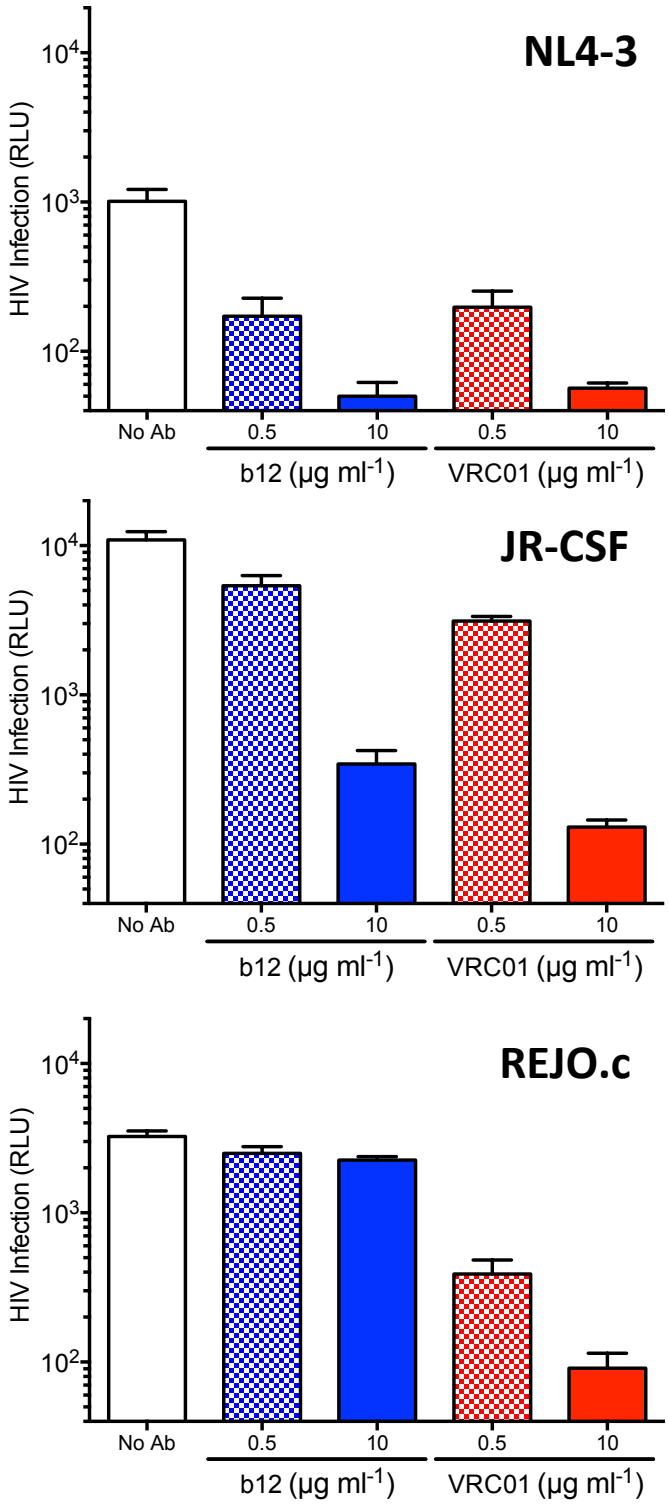

Figure S1 – In vitro susceptibility of HIV strains to b12 or VRC01 antibodies

TZM-bl cells were infected with the indicated strain in the presence of 0, 0.5 or 10µg/mL of the indicated neutralizing antibody for 48 hours prior to the addition of luciferin substrate and quantitation by luminometry. REJO.c strain shows resistance to high levels of b12 antibody but susceptibility to VRC01.

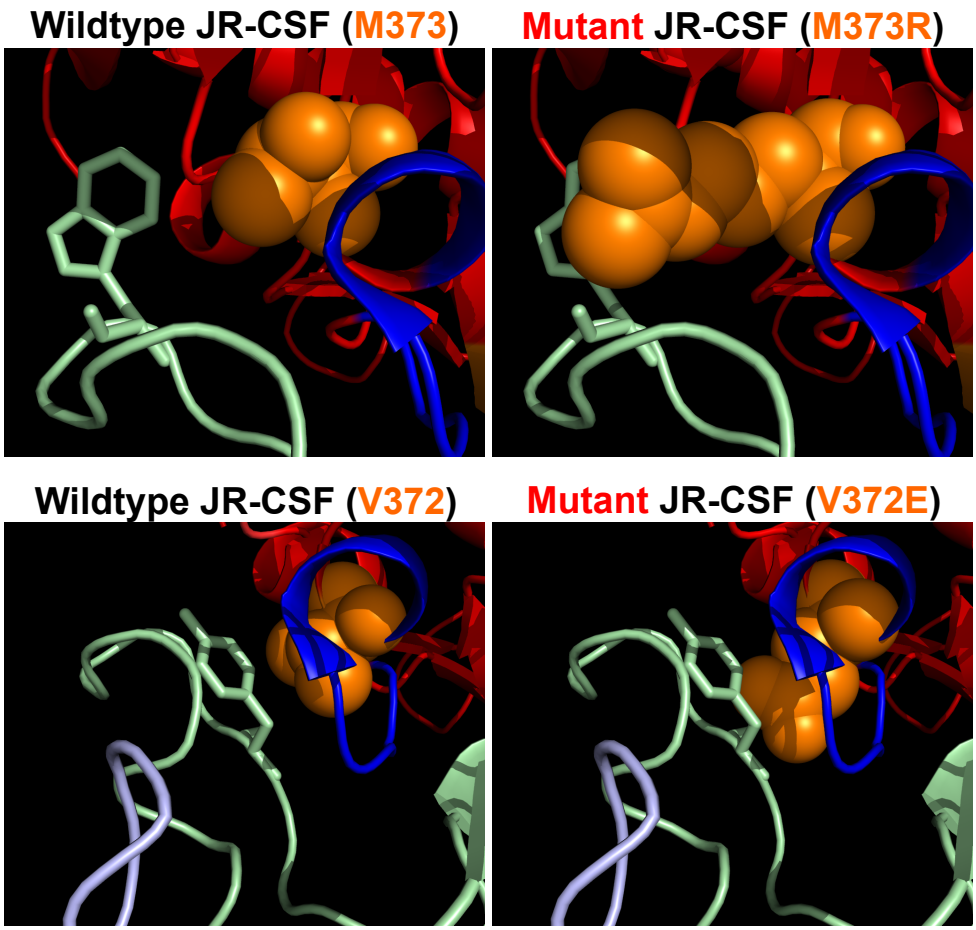

Figure S2 – Escape of JR-CSF from the b12 neutralizing antibody in humanized mice
Hypothetical structures of b12 heavy chain (green) interacting with gp120 (red) envelope protein with CD4 binding loop highlighted in blue and mutated residue highlighted in orange. Structures created in PyMol beginning with the 2NY7 structure and using the integrated mutagenesis tool.

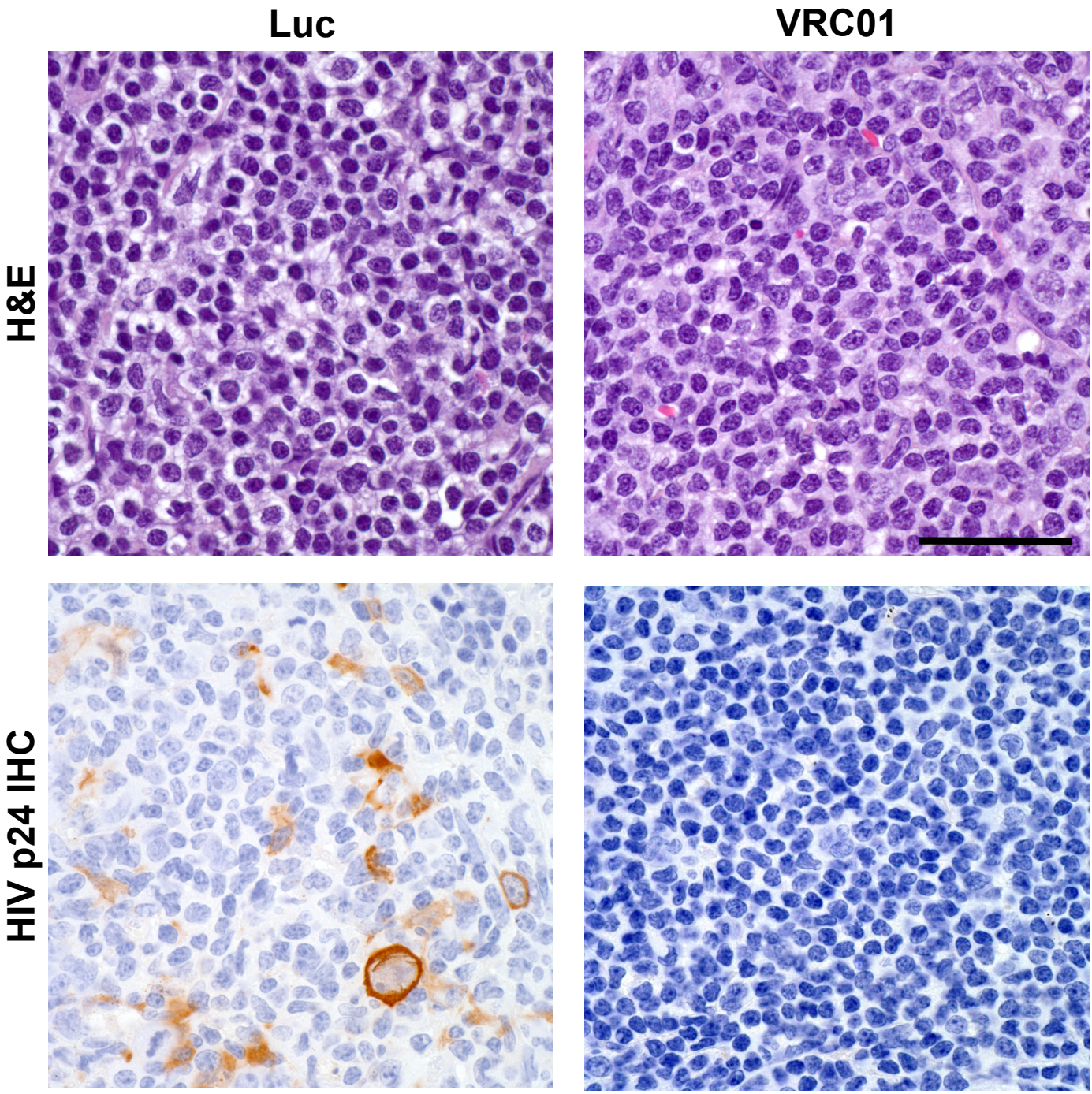

Figure S3 – Splenic infection following intravaginal challenge of BLT humanized mice with CCR5-tropic JR-CSF HIV

(top) Hematoxylin and eosin (H&E) staining of sections taken from paraffin-embedded spleen tissues from BLT mice expressing either luciferase (left) or VRC01 (right) that were intravaginally challenged 15 times with JR-CSF. (bottom) Immunohistochemical staining of HIV p24 antigen in paraffin-embedded spleen tissues from BLT mice expressing either luciferase (left) or VRC01 (right) that were intravaginally challenged 15 times with JR-CSF. Scale bar represents 40μm.

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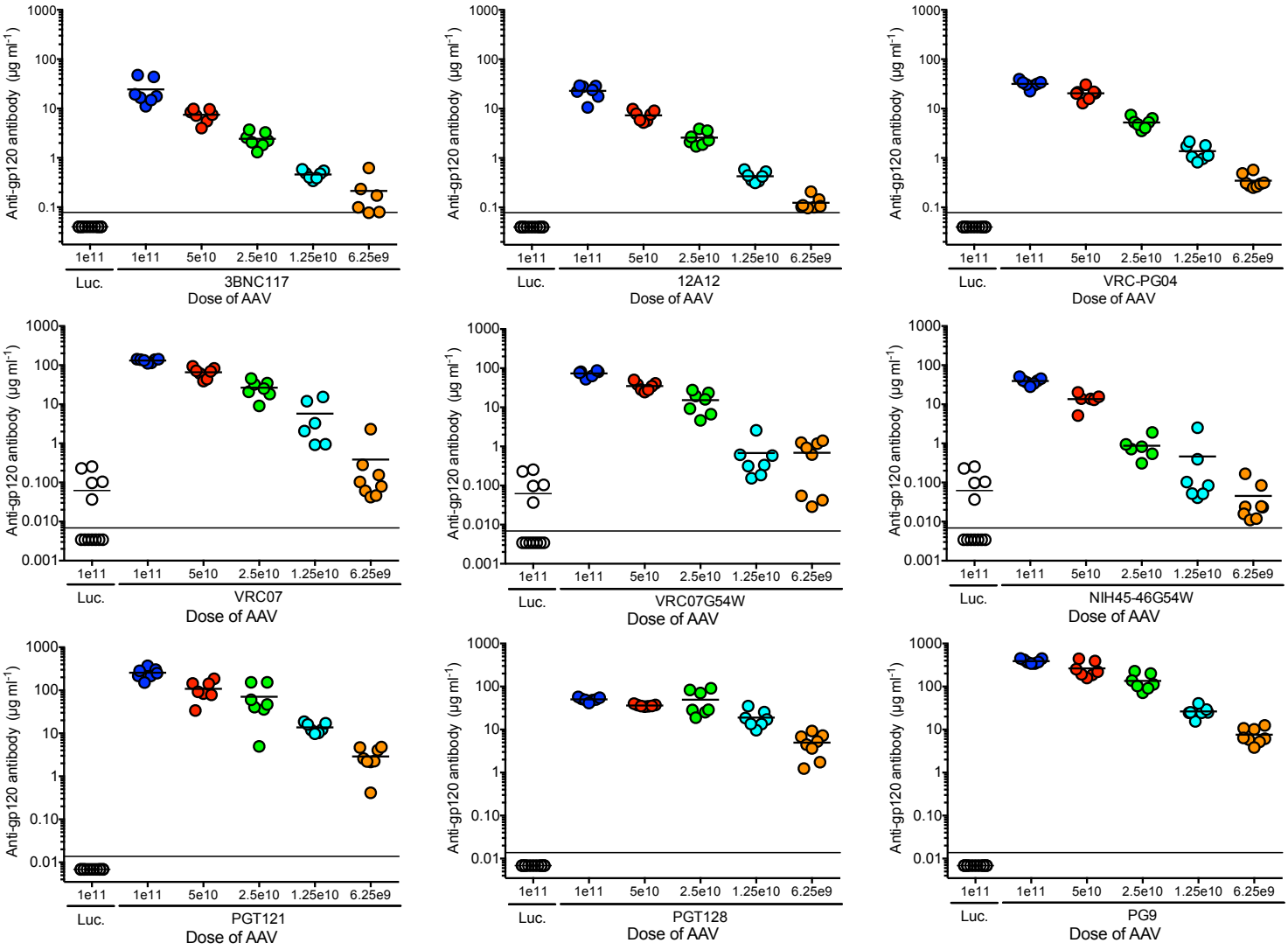
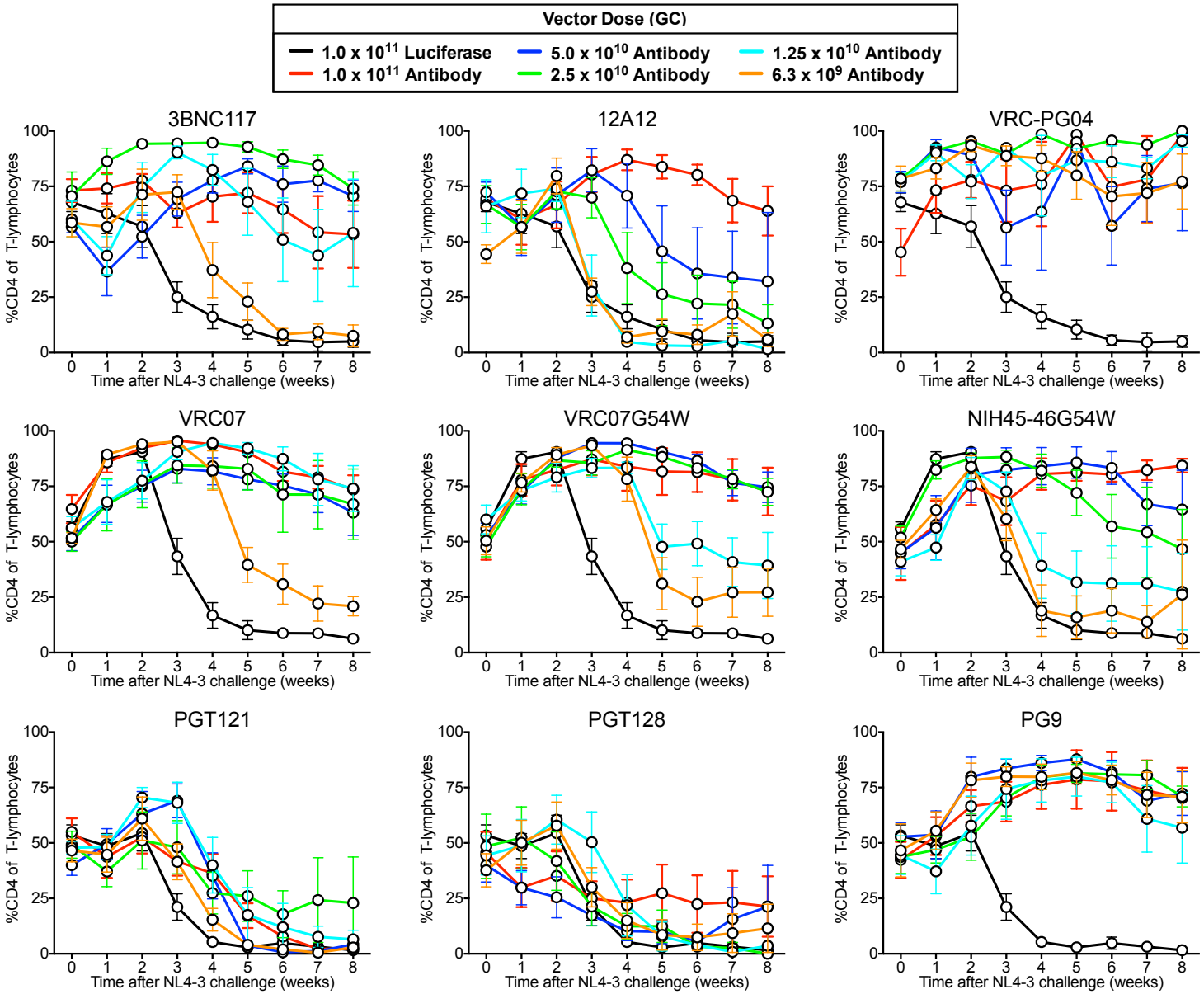


Summary of mean antibody expression in serum prior to HIV challenge (µg ml^{-1})

Vector	Dose administered (GC)				
	1x10^{11}	5x10^{10}	2.5x10^{10}	1.25x10^{10}	6.25x10^{9}
3BNC117	24.41	7.45	2.43	0.46	0.21
12A12	22.87	7.30	2.59	0.43	0.12
VRC-PG04	31.64	20.49	5.25	1.38	0.35
VRC07	131.42	65.82	26.51	5.76	0.39
VRC07G54W	73.51	35.00	15.30	0.68	0.69
NIH45-46W	39.24	13.58	0.87	0.46	0.05
PGT121	256.19	108.51	70.60	13.70	2.89
PGT128	50.34	36.26	49.46	18.97	4.96
PG9	390.61	263.42	134.81	26.32	7.58

Figure S4 – Expression of broadly neutralizing antibodies following titrated AAV administration
Concentration of gp120-binding antibody detected in serum of mice 5 weeks following administration of the indicated dose of VIP vector expressing each of the indicated broadly neutralizing antibody or luciferase protein. A single group of mice receiving luciferase served as controls for each of the mice receiving the same batch of expanded PBMCs (three antibodies tested per batch). Mean concentrations are summarized in the table below.



Protection against NL4-3 challenge in vivo by the indicated antibody concentration (μg ml^{-1})

Vector	Dose administered (GC)				
	1x10^{11}	5x10^{10}	2.5x10^{10}	1.25x10^{10}	6.25x10^{9}
3BNC117	24.41	7.45	2.43	0.46	0.21
12A12	22.87	7.30	2.59	0.43	0.12
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VRC07G54W	73.51	35.00	15.30	0.68	0.69
NIH45-46G54W	39.24	13.58	0.87	0.46	0.05
PGT121*	256.19	108.51	70.60	13.70	2.89
PGT128*	50.34	36.26	49.46	18.97	4.96
PG9	390.61	263.42	134.81	26.32	7.58

Blue: Protected, Yellow: Partial protection, Red: Not protected; * Antibody demonstrated no neutralization activity in vitro against NL4-3 strain,

Figure S5 – Minimum protective dose in vivo of broadly neutralizing antibodies against HIV
Peripheral blood CD4 cells as detected by FACS following intravenous challenge with 10ng p24 of NL4-3 HIV into mice expressing the concentrations of antibody as summarized in the table below. Table colors reflect complete, partial or no protection of CD4 cells following challenge. Concentrations listed in the table represent mean concentrations of serum antibody in challenged mice.

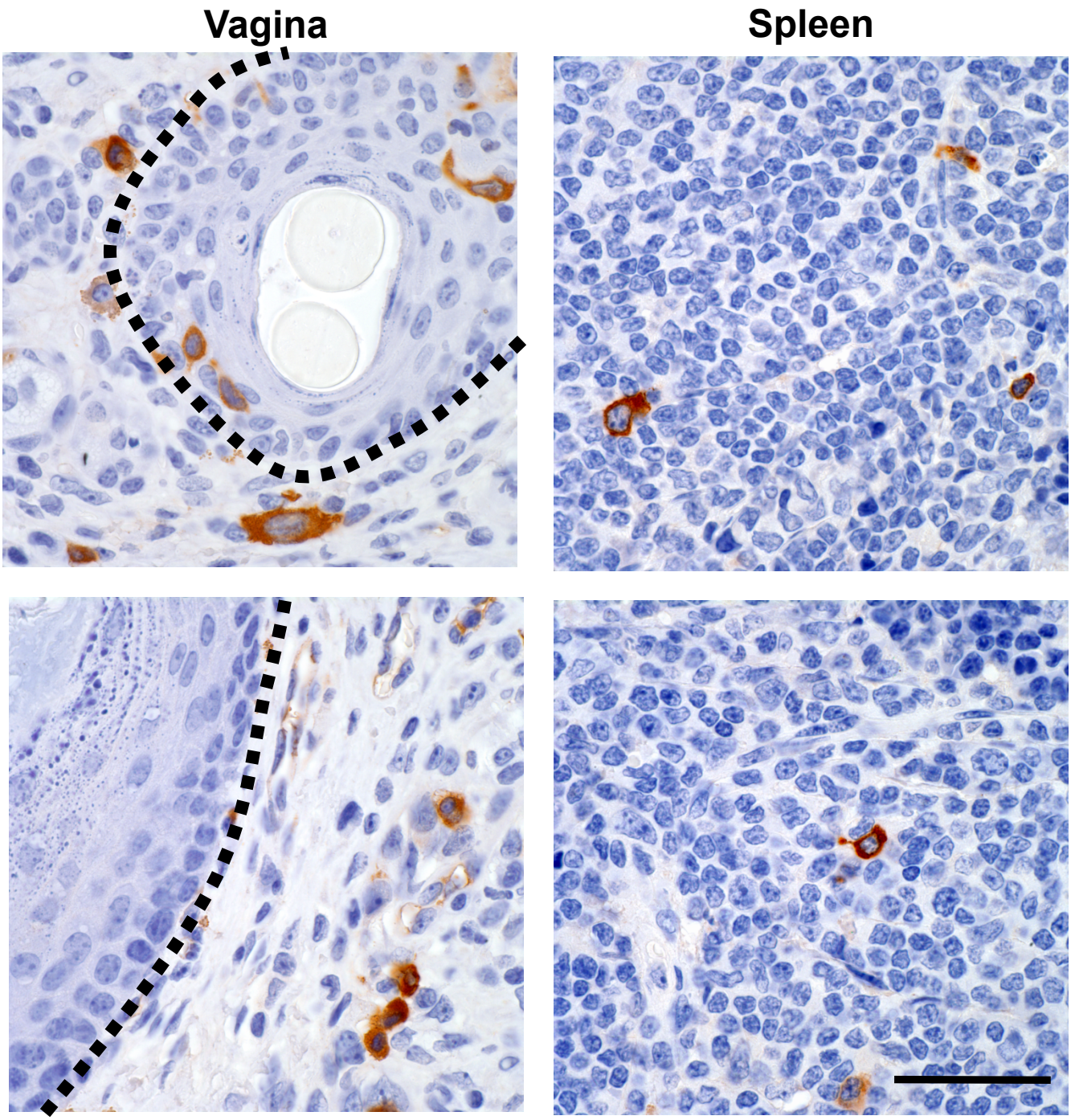

Figure S6 – Vaginal and splenic infection following intravaginal challenge of BLT humanized mice with transmitted founder HIV

Immunohistochemical staining of HIV p24 antigen in paraffin-embedded vaginal (left) or splenic (right) tissues from BLT mice expressing luciferase that were intravaginally challenged with REJO.c. Dashed line represents interface between epithelium (left) and lamina propria (right) demonstrating infected lamina propria lymphocytes. Scale bar represents 40μm.

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